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SURVEY OF CONTAMINATION IN FUEL TANKS OF DD-963 CLASS SHIPS.(U)
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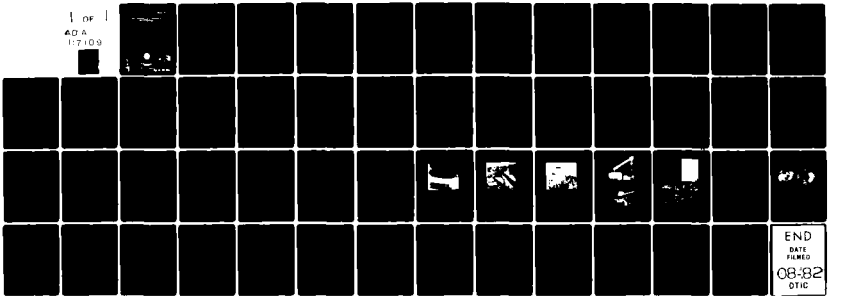

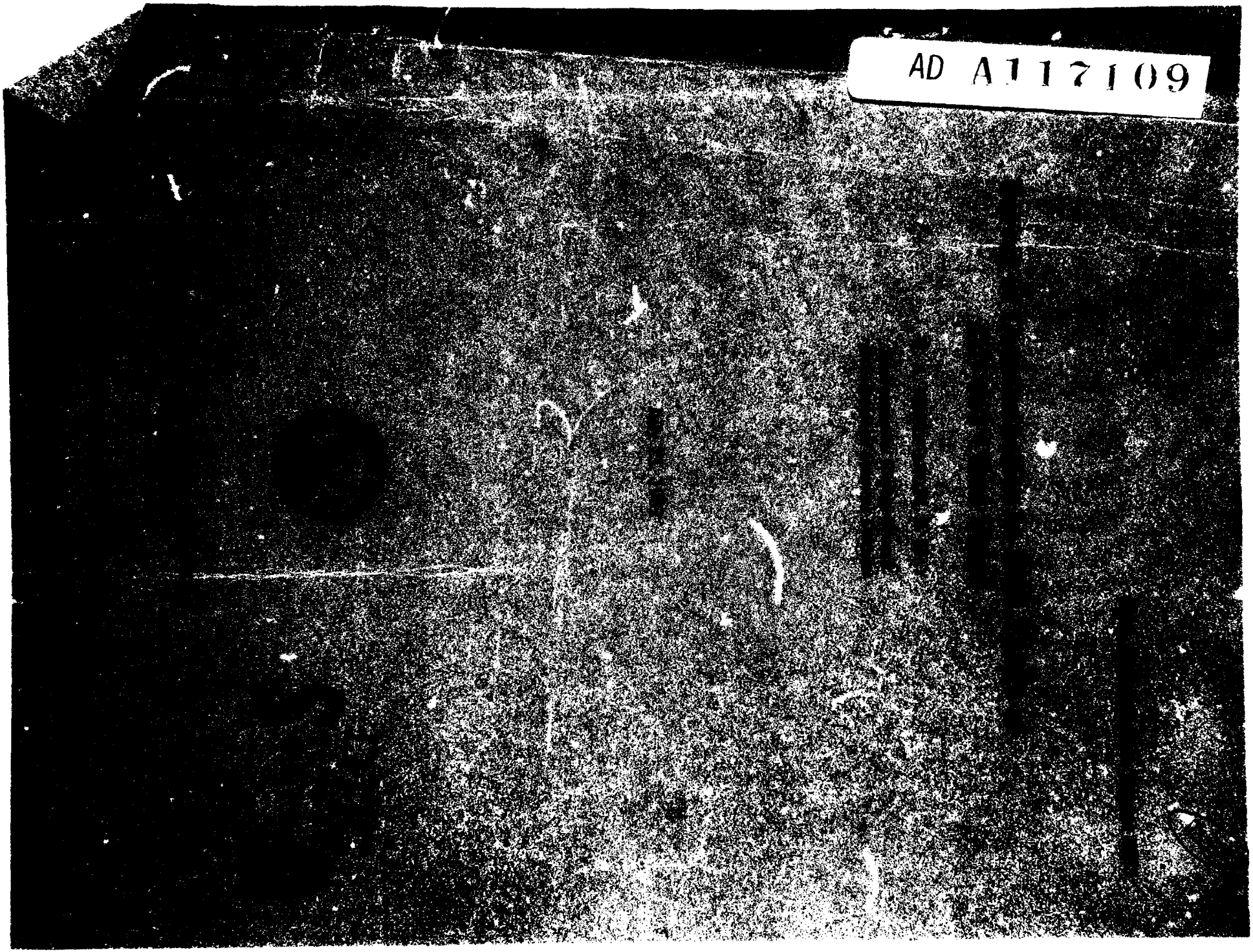

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Because of fuel contamination problems occurring on ships with water-compensated fuel tanks, a survey has been made of sludges collected from tanks on DD-963 class ships to assess sources of particulate matter, especially that generated by microorganisms.

More than eighty tanks on eight different ships were sampled and examined microscopically for the presence of microorganisms and other debris.

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20. ABSTRACT (Continued)

Assays for viable fungi, yeast and bacteria, including sulfate reducers, were made. Centrifugal fractionation separated the fuel and aqueous phases of the sludges and allowed the volumes of low-density particulates (lighter than water) and sediment to be estimated. The pH and salinity of the aqueous phase were measured and analyses made for organic matter, sulfide and metallic elements in the particulate matter.

There were considerable variations in amount, quality and microbial content of the sludges in different tanks even on the same ship. Viable microorganisms were always found but the dominant genera differed considerably. A high aqueous pH and the presence of sulfide were usually correlated with active sulfate-reducing bacteria and a low aqueous pH was always associated with high yeast and fungal content. In some tanks, including service tanks, fungal material made up a substantial portion of the sludge. Nevertheless, it appeared that well maintained centrifugal purifiers could keep the total volumes of sludge accumulated in service tanks at acceptably low levels.

An ubiquitous particulate contaminant found in all sludges, especially conspicuous in the low-density fraction, was of petroleum origin and is believed to come from oxidation of unstable constituents in diesel fuel. This material was also found on plugged coalescer filters. Much of the reported difficulty with excessive plugging of coalescer filters appeared to be due to taking on off-color fuel containing this kind of petroleum-derived particulate matter. Thus it appears that elimination of microbial growth in fuel storage tanks by the use of a biocide or other agents would not, under present conditions, affect fuel problems caused by this contaminant.

The role of seawater, metals and microorganisms in generating particulate matter by interactions with fuels, especially those prone to instability, needs further investigation.

CONTENTS

SURVEY OF CONTAMINATION IN FUEL TANKS OF DD-963 CLASS SHIPS

INTRODUCTION

Problems from microbial contamination of hydrocarbon fuels carried on naval ships have arisen in a number of instances over the years (1,2,3). In the 1960's aviation gasoline and jet fuel on carriers and LPH's became corrosive from fuel-soluble sulfides generated from the reduction of sulfate in seawater bottoms of storage tanks by anaerobic bacteria (1,4). Destroyers (1040-class) experienced plugging of coalescer filters and other operational problems from bacterial and fungal growth in water-displaced tanks carrying JP-5 (5). More recently on gas turbine powered ships using diesel fuel, rapid accumulation of particulate matter in centrifugal purifiers and coalescer filters has occurred (6,7,8). The Royal Navy of the U.K. has experienced acute fuel flow blockages in their gas turbine powered frigates and destroyers due to microbial growth and has instituted a comprehensive program to assess the dimensions of the problem and to evaluate biocides and alternative methods of controlling microbial growth (3).

Ships employing water-compensated fuel tanks have been especially vulnerable to particulate contamination (Fig. 1). In ships of the DD-963 class, increases in fuel particulate load have been detected after the fuel was taken aboard (9). The possibility that microbial contamination might be an important contributor to such contamination was recognized in the design of the DD-963 class ships by including equipment for injecting a fuel-soluble, organo-boron biocide into the fuel as it was brought aboard. Unfortunately this is an ineffective biocide where large amounts of water are present, as in water-compensated tanks, and its use is no longer required (10,11). Uncertainty about the sources of particulate matter in fuel on DD-963 class ships has prompted a request for an investigation to ascertain whether microbiological contamination is now or is likely to become significant and for a proposal of appropriate methods for dealing with such contamination if needed. A consensus was reached among representatives from NAVSEA, NAVSSES and NRL that such a study should be carried out and a project was funded for FY-81 to allow NRL to execute it (12,13).

The determination of whether microbiological contamination is a real or potential source of substantial fuel contamination in a ship's fuel system depends heavily on the methods used for taking samples. Obtaining a sample from a water-compensated fuel tank which carries a particulate load representative of the entire tank is impossible under normal circumstances. Tanks can be sampled through a stripping tube terminating a few inches above the bottom of the tank. Fuel, water, or both, could be obtained in this way depending on the status of the tank at the time (see Fig. 1). Concentrations of particulates might be high after stirring, e.g., after refueling, rough weather, or sharp turns, and low if the ship has been in port and quiescent for some time - unless a sludge has settled around the bottom of the stripping tube in which case a very high particulate load would

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be found. For these reasons sampling was confined to the material left in fuel storage tanks after they were drained in preparation for cleaning or repair. In this way the quantity and character of the accumulated sludge could be evaluated and the condition of the tank surfaces inspected. It was assumed that this sludge contained material representative of that which becomes entrained in fuel as it flows toward the engine. It should be recognized, however, that material normally present at the fuel/water interface may have been removed from the tank during drainage.

In this investigation, sludge samples were taken by NRL personnel or under NRL supervision whenever possible. At other times ship or shipyard personnel obtained samples after instructions from NRL. Schematic representations of the fuel system and tank arrangement are given in Figs. 1 and 2. A summary of the ships, times and number of tanks sampled, and the personnel involved is given in Table 1. Ships listed for 1979-80 were sampled because of reported problems before the present program was started and have been included for comparison with "normal ships" not reporting problems. It was learned that three ships included in the 1981 survey had recently experienced periods of rapid coalescer filter clogging which were not investigated. Nevertheless, all of the tanks in the 1981 survey have been taken as a group to represent the average condition for ships of this class.

MATERIALS AND METHODS

Sample Collection

Sludge was scooped into clean polyethylene sample bottles from tank bottoms. In the case of NRL-collected samples, a small plastic scoop was used to remove sludge from the bottom, sides or other tank surfaces. The sample was then deposited into a sterile, polyolefin sample jar provided with a screw cap. NRL-collected samples were returned to the laboratory for analyses within 1-3 days. Transit times for other samples varied from 1 to 4 weeks.

In a few cases the surfaces of cleaned tanks were sampled by scraping or swabbing with cotton. Fuel level gauge guides were similarly sampled.

Water samples were taken in sterile plastic bottles at dockside locations where ships were tied up at the San Diego and Norfolk Naval Bases and at the Ingalls shipyard at Pascagoula, MS.

After arrival at the laboratory, samples were kept at $4^{o}C$ except when portions were being removed for analysis.

Microscopic Examination

Phase contrast microscopy was used to detect motile bacteria and fungal hyphae (filaments) as soon as the samples arrived in the laboratory. The presence of protozoa, algae and any unusual type of debris was also noted. The large amount of particulate matter present often made it very difficult to distinguish and identify smaller microbial particles such as non-motile bacteria and spores. Epifluorescent microscopy of preparations treated with fluorescent stains greatly improved the visibility of microorganisms in these cases.

Viable Aerobic Microorganisms

Sludge samples were streaked on agar plates of the following media: Marine Agar (2216, Difco) for aerobic marine bacteria; Tryptone Yeast Glucose Agar (Difco) for non-marine bacteria and yeasts; Potato Dextrose Agar (Difco) with 0.05% yeast extract added for fungi. A conventional 3-mm loop was used to remove sludge from the sample jar and to streak the plates. If the sample was mainly fuel or water, then 1 ml was spread on each plate. The plates were incubated at 25° C and were read after 3 days, 1 week, and longer if necessary. The amount of growth was classified as detectable (+) or heavy (++). Types of growth were noted and tentative identifications were made of the yeast and fungi.

Viable Sulfate-Reducing Bacteria

The sludge was analyzed for anaerobic sulfate-reducing bacteria by placing 0.2 ml of the sludge or 1 ml of water or fuel, depending on the sample type, into 10 ml of Sisler and ZoBell triple strength medium (14,15) in 16 x 125 mm screw-capped tubes. Approximately 3 cm of sterile mineral oil was added on top of the medium to help maintain anaerobic conditions. The tubes were incubated at 25° C. The activity of the sulfate-reducing bacteria was estimated from the rapidity with which the medium blackened as a result of the reaction of microbially generated sulfide with the ferrous iron in the growth medium to form black ferrous sulfide. An active sample produced complete blackening of the medium in 2 to 3 days.

Enrichment Cultures

To determine whether or not the microorganisms present in the samples were capable of growing on a fuel/water system, 0.2 ml of sludge or 1.0 ml of water, was placed in 10 ml of aqueous medium under 10 ml of sterile diesel fuel (DFM) or jet fuel (JP-5). Four types of aqueous media were used: aged seawater with 0.05% peptone and 0.05% yeast extract added; Klausmeier mineral salts mixture as modified by Park (16); Bushnell-Haas (17) mineral salts; and Bushnell-Haas mineral salts with the dipotassium phosphate removed. The removal of the phosphate from the latter medium lowered its pH from 6.5 to approximately 5.

The three mineral salts media were essentially fresh water media and contained no carbon sources. Incubation was at 25° C and the tubes were observed over a period of several months. Some of the resultant growths were examined microscopically for tentative identifications.

Centrifugal Fractionation

About 22 ml of sludge sample homogenized by vigorous shaking were centrifuged in a 25 ml Corex (Corning) tube at 15,000 x *g* for 15 min in a swinging bucket rotor. The lengths of the centrifuge tube occupied by the sediment, water, low-density particulates and oil were measured with a cathetometer and the volumes of the fractions were obtained from a calibration graph (Fig. 3). The fuel layer was discarded and the water layer under the low-density particulate fraction removed by Pasteur pipette and retained. The low-density particulates and sediment were washed separately with intervening centrifugal separations in two 10-15 ml portions of distilled water to remove salt and two 10-15 ml portions of petroleum ether to remove residual fuel.

Chemical Measurements

The water phase collected after centrifugation of the sludge was checked for pH with a combination glass-reference electrode and for salinity by refractometry.

The sediment and low-density particulate fractions were transferred to tared porcelain crucibles and dried at 100° C to constant weight and then ignited at 500° C overnight in a furnace followed by heating over a Meker burner at about 900° C to constant weight. The difference in ignited and dry weights was taken as a measure of organic matter in the sample.

Elemental metal analyses were made on ignited samples by DC Argon Plasma Atomic Emission Spectrometry according to procedures described in Appendix A.

Sludge samples or particulate fractions were analyzed for sulfide by warming a small amount of the acidified material in a test tube covered with moist lead acetate paper.

RESULTS

General Observations

More than eighty tanks were sampled on eight different ships. Major results are summarized in Tables 2-11. Where it was possible for NRL personnel to judge the condition of the tanks, a rating of the amount of sludge is given (Tables 8-11). These ratings are exemplified by Fig. 4-a,b,c.

There was great variation in the amount of sludge present in the storage tanks of a ship and no consistent correlation of the amount with the position of the tank in a bank. Service tanks generally had less accumulated sludge than storage tanks as would be expected after the fuel had been centrifugally purified (see Fig. 1). An unusually heavy accumulation of sludge of 4-6 inches was reported to have been found in receiving tanks 6-260-1 and -2 on DD-967 (Table 11).

Corroded areas were occasionally seen in crevices, on edges, pipe hangers, etc., especially in expansion tanks. The DD-968 was reported to have heavy fuel tank corrosion, particularly in the expansion tanks and several storage tanks in aft and forward banks (18). This ship had not previously been provided with zinc anodes, however, and their installation during overhaul was expected to provide relief from this problem.

The appearance of the sludge varied somewhat but was generally black, dark brown or olive in color. Material from expansion tanks was sometimes dark red because of rust and occasionally a sludge was dark tan due to sand and silt. Sludge consistency varied from slimy and jelly-like to coarse and granular (Fig. 5). Well-drained sludges with considerable inorganic material had salt-free, non-volatile solids contents of 10% or more after drying at 100°C, but more typical values were less than 3%.

Microscopic Examination

Most of the sludge samples contained a variety of particulate matter along with emulsified fuel and water droplets. Large amounts of fungal contamination were readily recognized by the characteristic appearance of mycelial filaments (Fig. 6-c), but bacteria were difficult to distinguish unless they were motile or treated with a fluorescent stain (Fig. 6-a,-b)

A particulate material common to most sludge samples was brown to black in color, had a density less than that of water, was soluble to a considerable extent in a mixture of equal parts of toluene, acetone and methyl alcohol ("triple solvent"), and thus appeared to be of petroleum origin (19,20,21). In diesel fuel samples which had become off-color because of inherent instability, the particles were 1-4 μm in diameter, approximately spherical in shape, and were aggregated to various extents as shown in Fig. 6-d. Much more heterogeneous particulate matter was found in fuel samples suspected of being contaminated by petroleum residue from land-based tanks previously used for storing black oil. The pleated paper portion of coalescer filters reported to be plugged held considerable deposits of material soluble in triple solvent. Thin-layer chromatography and liquid chromatography were used in some experiments in an attempt to characterize the material solubilized by triple solvent (22).

Centrifugal Fractionation

Centrifugal fractionation provided a means of separating and clarifying water and fuel phases and of making estimates of the volumes of low and high-density particulates present. It is likely that the low-density particulates are least efficiently removed by a ship's centrifugal purifier. A representation of the fractions present in the centrifuge tube is shown in Fig. 3. The high-density fraction generally contained clay, sand, rust, petroleum residue, microbial debris, etc. and had an organic content of 3-76%. The low-density fraction had a greasy consistency, a higher average organic content (36-100%), as might be expected for material less dense than water, and consisted largely of the particulates from petroleum as described above. These fractionations were reproducible for a given sludge but were not clean cut separations of low- and high-density constituents. Resuspension of the separated particulate fractions by sonic treatment in water and petroleum ether mixtures and recentrifugation showed that each of the initial fractions contained a small amount of the particulates from the other. The fractionation apparently depends on the state of aggregation of the low- and high-density particles and the resultant density of the aggregate. The presence of inorganic material in the low density fraction (Tables 2,3,6-11) could be explained by a similar mechanism since it would generally be expected to have a density exceeding that of water. In some cases most of the viable microorganisms were associated with the heavy sediment while in others the same spectrum of organisms appeared in both sediment and low-density fractions. Again the state of aggregation of the low-density particulates with microorganisms probably determined this distribution.

Microbiological Findings

Viable microorganisms were found in all samples except for one which was 100% fuel. The most prevalent genera are listed in Table 12 in the order of observed frequency. All are recognized as typical hydrocarbon contaminants encountered in fresh as well as salt water systems (2,16,23). Algae and protozoa were occasionally observed in the water phase of the samples. These organisms were undoubtedly admitted with seawater ballast and, since they have no capability for growing in the fuel tanks, are not recorded in the results. The gross appearance of the sludges was not a reliable guide to the amount or kind of microorganisms present, although a stringy, fibrous consistency was usually correlated with the presence of a high content of fungal hyphae.

Distinct differences appeared in the kind of microbial species present in different tanks. Sulfate-reducing bacteria occupied one extreme and fungi and yeast the other. Where sulfate-reducing bacteria were very active, there was little or no microscopic evidence that fungi or yeasts were present in an actively growing condition but, since they could be cultured on favorable growth media, were probably present as dormant

spores. Other tanks were dominated by actively growing fungi and yeasts and sulfate-reducing bacteria were generally absent.

Significant correlations exist between the chemical and microbiological results and are summarized in Table 13. Above pH 7.8 bacteria were dominant; sulfate-reducers were common and frequently accompanied by positive tests for sulfide. A wide variety of fungal species was present but relatively few yeasts. Between pH 7.8 and 4 fewer bacteria were found but yeasts (mainly Candida) and a variety of fungi (including Paecilomyces and Cladosporium resinae) predominated. Viable sulfate reducers were sometimes present but rarely sulfide. Below pH 4 bacteria were rare and fungi and yeasts were numerous but the variety tended to be restricted to C. resinae and Candida. Differences in viable microbial varieties present in these sludge categories are illustrated in Fig. 7. The sludges from about one-half of the tanks examined could be readily categorized as dominated by sulfate reducers, about a third of the tanks were dominated by yeast and fungi and the remainder were in an intermediate condition (pH 4 to 7.8). A low aqueous pH can be taken as a good diagnostic indication of heavy fungal contamination.

Salinity did not appear to be a controlling variable so far as microbial growth was concerned. Salinities above 37 ppt exceed that of normal seawater and could be due to soluble ionic constituents extracted from sludge and fuel. The low salinity in the purifier sample of DD-986 (Table 6) indicates an intrusion of fresh water. Unexplained low salinities in service tanks were also occasionally found on other ships (Tables 3,9,11).

The analysis of microbial composition of harbor waters where DD-963 class ships were docked showed that a variety of bacteria were always present, including sulfate-reducing bacteria (Table 14). A viable yeast sometimes appeared but the fungus, C. resinae, was never found. Fuel samples, on the other hand, typically contained viable C. resinae but no bacteria or yeast (24,25). Cladosporium resinae in fuel exists mainly as dormant spores which can germinate and grow when they encounter a favorable environment. Thus it appears that bacteria and yeast are introduced into the fuel with the ballast water and a major fungal contaminant enters with the fuel.

Table 15 lists those organisms present in sludges from ship tanks which were capable of growing in a water/fuel system (enrichment cultures). There is little doubt that the predominant species of fungi and bacteria found in a viable condition in fuel tanks are also able to proliferate there. In seawater the growth of fungi tends to be slow unless the pH is lowered by at least one unit and some nutrients are present, e.g., yeast extract and peptone. Other experiments have shown that C. resinae grows better with slightly polluted coastal seawater than with open ocean water. These observations agree in general with those of Kuo who has shown that the presence of

undefined growth factors (e.g., in dust) stimulates growth of yeast in fuel/water systems (26). In a recent extensive study by Turner the effects of organic and inorganic nutrients on the growth of C. resinae have been more precisely defined (27).

Fuel Level Gauge Malfunction

Personnel on most of the ships involved in this study were asked about past experience with fuel level gauges in the fuel tanks. In every case these gauges were considered unreliable. A possible reason for this is that the floats do not move freely on their guides. Guides in the tanks examined by NRL personnel appeared free of corrosion and any substantial amounts of adhering material. An examination of the gummy residue on the guides showed an intimate mixture of petroleum degradation products and fungal fragments as shown in Fig. 8. The rheological properties of this mixture are unknown but might be relevant to gauge malfunction.

Metallic Elements in Incinerated Sludge

Major metallic elements present in incinerated sludge samples are summarized in Table 16 and the detailed results for nineteen elements are given in Appendix A. A major element not recorded was silicon because sand particles were not completely dissolved by the digestion procedure. Carbon, sodium, and tungsten were also present.

Iron was the dominant metal present in both sediment and low-density particulates in nearly all cases. It was particularly high in the service tanks of the DD-986. The presence of metallic compounds in the low-density particulate fraction is another indication that material more dense than water can be buoyed up in aggregates of petroleum-derived particulates. The principal sources of iron, copper, nickel and zinc are likely to be incompletely coated tank surfaces, piping and sacrificial anodes.

DISCUSSION

Microbial Ecology of Fuel Tanks

No sterile fuel tanks were found in this investigation and with present operating procedures on ships with water-compensated systems, sterility is impossible. Even after cleaning the tanks, there are viable yeast and fungi on the tank surfaces which can grow when the tanks are filled again (Table 5, tank 5-260-2). Fresh fuel is likely to contain fungal spores and the first admittance of ballast water will introduce additional organisms and a favorable environment for microbial growth, especially if the water is from a coastal or harbor area. Results from enrichment cultures made in this investigation show that typical fuel contaminants are capable of growing under the conditions found in a fuel tank (Table 15). It is of interest to know what

variables control the direction of microbial dominance in a fuel tank after it is inoculated with microorganisms because the information might be useful in devising new methods for control of the most troublesome organisms. In the absence of data on the changing conditions and microbial content of fuel tanks with time, nothing definitive can be said about this, however, several pieces of evidence bear on the question of microbial dominance in important ways.

An obvious variable which could control microbial species dominance in a fuel tank is the relative concentrations of different microorganisms in the fuel or water admitted to the tank. Ballast water containing sediment and high bacterial counts would favor the proliferation of sulfate-reducing bacteria in the sludge whereas low bacterial counts in the water and relatively high fungal counts in the fuel would favor the dominance by fungi.

There are also important interactions among the different species of microorganisms. In spite of the common occurrence of the fungus, C. resinae, in contaminated fuel tanks, it has been found that the organism does not grow well in pure culture in seawater media (28). A laboratory investigation of this anomaly showed that the pH of seawater ($\sim$ 8.0) was too high for C. resinae growth (28,29). In the presence of other organisms, particularly yeasts, which were able to grow sufficiently to generate acidic metabolites and lower the pH slightly, it was possible for C. resinae to initiate growth. It is significant that yeasts were always present in the sludge samples whenever substantial amounts of C. resinae were found and that the measured pH was always in a favorable range for the growth of this fungus. This indicates that the conclusions drawn from the laboratory experiments to establish the basis of the favorable interaction of yeast on the growth of C. resinae in seawater are also valid in seawater ballasted fuel tanks. These findings may be worth exploring from the point of view of controlling fungal growth by maintaining a high aqueous pH.

The availability of oxygen might also be decisive in directing microbial growth in a sludge. If aerobic bacterial activity is high, oxygen could be depleted and fungal growth inhibited in an environment already having an unfavorably high alkalinity. If anaerobic sulfate-reducing bacteria then become established, the presence of traces of toxic quantities of hydrogen sulfide might further inhibit the fungi. On the other hand, if aerobic bacteria are few, fungal development may begin at least in localized areas, and as the organic acid products of fungal hydrocarbon metabolism are released, the pH would decrease and permit accelerated growth. A low pH would then generally be expected to inhibit bacterial growth. Alternatively, the microbial dominance might be directed by the degree of oxygenation of the sludge as altered by compaction and agitation which would affect oxygen diffusion into the sludge from the fuel and water phases.

Active sulfate-reducing bacteria were detected in about half of the samples examined. Sulfides were also detected often but no samples showed more than a trace of volatile hydrogen sulfide although an unconfirmed report of detectable amounts of hydrogen sulfide in tanks on one ship was received. The sulfide being detected in the samples must exist almost entirely in combination with metals, especially iron. In view of the prolific generation of hydrogen sulfide by sulfate reducers in Avgas tanks on carriers 15 to 20 years ago, the question arises as to why this is not also a problem in diesel fuel tanks on ships today. Only speculative answers can be offered. The fuel and water may be turned over rapidly enough that the anaerobic conditions required for sulfate reduction are restricted to protected layers of sludge. The coating on tank surfaces of DD-963 class ships may restrict available iron which has been cited as a stimulant of sulfate reducer growth (30,31,32,33). There may be subtle differences under field conditions between diesel fuel and Avgas as far as their effects on sulfate reducer growth is concerned but experiments in our laboratory have revealed none.

Though sulfate-reducing bacteria do not appear to be a significant contributor to fuel contamination aboard DD-963 class ships, it should be kept in mind that corrosion may be enhanced under stagnant sludges infected with these bacteria and under warm conditions the growth of these bacteria may be stimulated sufficiently to generate significant amounts of fuel soluble sulfur that could become a factor in turbine blade corrosion (34).

Microorganisms and Sludge

Sediment tends to form spontaneously in stored distillate fuels as a result of complex oxidative reactions (21). There are reports that the presence of water, metallic elements and organic acids can accelerate sediment formation (35,36). Cole and Nixon have shown that iron in the presence of seawater increases the generation of insolubles in fuel, especially in those fuels which have been cracked (35). The results in such studies depend on the composition of the fuel and the presence of trace constituents, especially heterocyclic nitrogen compounds (36). Growing microorganisms may introduce additional variables which should be considered. For example, fungi are known to oxidize fuel hydrocarbons to fuel-soluble organic acids which could accelerate sediment formation (27,37).

In addition to their physical presence, microorganisms may also contribute to sludge by releasing surface active materials that may induce and stabilize emulsions (37,38,39). It has been claimed that the use of cationic surfactants with biocidal properties greatly reduced the rate of sludge formation in fuel oil tanks on ships (40). Unfortunately no systematic study of such effects has been made that could be reliably applied to ships with water-compensated fuel tanks.

In a number of instances in this study the volume of sludge material occupied by fungal and yeast mycelia was judged by microscopic examination to be substantial. Mycelial strands were tangled together with other debris in coherent clumps (Fig. 6-c). While the fungal growth in these cases would add to the load which a centrifugal purifier on a ship should remove, the binding together of a variety of small particulates might increase the efficiency of their sedimentation compared to the case with no fungi where the particles are more dispersed. No definite conclusion can be reached on this but high concentrations of fungi were certainly present in purifier sludge (DD-986) (41,42).

Sludges may also provide a protected environment for microorganisms. The properties of the water phase (and possibly the fuel phase also) are not likely to be the same as the bulk of the water (or fuel) in a tank. The pH of a large amount of ballast water, for example, is unlikely to be as low as that in some of the sludges sampled in this study. The pH lowering in the sludges will depend on the acid generating activity of the fungi there and the rate of exchange and neutralization of hydrogen ions in the sludge with the contiguous seawater phase. The sludge may thus be considered a microenvironment where microorganisms can generate chemical changes not characteristic of the tank contents as a whole. This might have significant consequences as far as the surfaces of the tank are concerned. Accelerated corrosion at imperfections in the coatings as a result of low pH or the activities of sulfate-reducing bacteria might be possible. The presence of a protected environment is also probably essential for sulfate reduction by bacteria because it is highly unlikely that the entire fuel and water content of a tank under active use could easily become sufficiently depleted in oxygen.

Should Microbial Growth in Water-Compensated Fuel Tanks Be Controlled with a Biocide?

In only one instance has a reported fuel contamination problem on DD-963 class ships been attributed in a major way to microbial growth (DD-986). In this case substantial amounts of water were present in samples from a purifier sump which led to suspicions that the purifier was not well maintained (6,7).

In other cases high rates of coalescer usage were attributed by ship personnel to taking on off-color fuel. The major contaminant in these off-color fuels, insofar as it was possible to examine them, was petroleum-derived. Problems with these fuels subsided as they were used, blended or off-loaded. In two cases where clogged coalescers were available for examination, petroleum-derived material appeared to be the major particulate deposit (DD-972, DD-986). It is not likely that any procedure which controlled microbial growth alone would have greatly alleviated these problems.

The weight of evidence at this point does not support the use of a biocide. These materials may present some hazard to ship personnel and may also have unfavorable environmental effects when they are off-loaded (43,44,45). A marginal improvement in fuel quality which may be achieved by eliminating microbial growth through the use of a biocide does not appear worth these risks and the additional expense. With the present mode of operating these ships, more important sources of particulate matter would appear to be the fuel insolubles formed by inherently unstable fuels and the array of particulates introduced with the ballast water.

Should microbial contamination of ship fuels increase sufficiently to cause a reexamination of the advisability of employing a biocide for control, there are aspects of the present investigation which should be taken into account in the choice of a biocide. The use of a biocide to treat a tank in which there is already an established sludge infected by microorganisms would not be effective because of the slow rate of diffusion of the toxin into the sludge mass where the organisms are most active. This conclusion has been borne out in both field and laboratory studies (46,47). A biocide (applied to a clean tank) which collects at oil/water interfaces might be a good choice for fungal control because of the tendency of these organisms to grow there (40,48,49). As sludge is formed, any oil and water emulsions generated would also include the biocide at the fuel/water interfaces. A disadvantage may be an increased stability of water emulsions in the fuel.

Minimizing Problems from Microbial and Other Fuel Contaminants

While it should be obvious from the foregoing that it is not practical to try to achieve sterility in a fuel tank except by introducing a toxic agent, still much can be done to minimize the microbial contribution to fuel tank contamination.

It has become clear during investigations of fuel contamination on DD-963 class ships that problems originated before the ships left the shipyard at Pascagoula, Mississippi because of the introduction of silt laden, polluted ambient water into the tanks (41). This imposed a burden on the centrifugal purifiers and immediately provided the ingredients for a sludge and a favorable environment for subsequent microbial growth. The initial use of a ballast water free of particulates would certainly have helped to eliminate fuel contamination problems encountered on new ships.

It is understood by personnel on some ships that taking harbor water into compensated tanks leads to subsequent fuel problems. The elimination of particulates from this water would help. Soluble nutrients would remain in polluted water, however, and these would probably accelerate microbial growth in the tanks to some extent (27).

There will obviously be occasions when a ship cannot avoid taking on particulate matter in the fuel or water and the on-board generation of particulates from unstable fuels is likely to continue. During this study, however, it was apparent that some ships can keep service tanks relatively free of water and particulate matter over considerable periods of time. This appeared to be directly related to proper maintenance and operation of the purifiers. This is not the case on all ships, however, because of mechanical failures in the purifiers, the tedious, time consuming job of cleaning the purifiers and the possibility of purifier disc damage during cleaning (6). There are also reported to be sources by which fresh water can enter the purifier and service tanks. This would explain the low salinities sometimes encountered in this study. This water may contain microbial nutrients and should be rigidly excluded.

The development of a self-cleaning purifier capable of continuous operation with a greatly reduced need for disassembly and cleaning may provide a major advance to keeping fuel and service tanks clean (50). The installation of a particle filter between the service tank and coalescers may then remove the low-density suspended material remaining in the fuel after centrifugation (51).

RECOMMENDATIONS

1. The importance of keeping purifiers clean and well maintained should be emphasized to ship personnel at every opportunity.

2. The evaluation of self-cleaning purifiers and of particulate filters for ships with water-compensated tanks should be accelerated.

3. The accumulation of water in service tanks should be minimized and taking on compensation water from harbors and other sources likely to be polluted or sediment laden should be avoided. This pertains especially to the construction and overhaul site at Pascagoula, MS.

4. The feasibility of controlling fungal growth in water-compensated fuel systems by maintaining high alkalinity in the water should be investigated.

5. The effectiveness of surface active biocides for inhibiting microbial growth and reducing sludge generation in water-compensated tanks ought to be determined (40).

6. A determination should be made of the extent to which the generation of solid particulate matter (microbial and petroleum-derived) is accelerated under the conditions present during fuel storage on ships with water-compensated tanks.

SUMMARY

Because of fuel contamination problems occurring on ships with water-compensated fuel tanks, a survey has been made of sludges collected from storage tanks on DD-963 class ships to assess sources of particulate matter, especially that generated by microorganisms.

More than eighty tanks on eight different ships were sampled and examined microscopically for the presence of microorganisms and other debris. Assays for viable fungi, yeast and bacteria, including sulfate reducers, were made. Centrifugal fractionation separated the fuel and aqueous phases of the sludges and allowed the volumes of low-density particulates (lighter than water) and sediment to be estimated. The pH and salinity of the aqueous phase were measured and analyses made for organic matter, sulfide and metallic elements in the particulate matter.

There were considerable variations in amount, quality and microbial content of the sludges in different tanks even on the same ship. Viable microorganisms were always found but the dominant genera varied considerably. A high aqueous pH and presence of sulfide were usually correlated with high sulfate-reducing activity and a low aqueous pH was always associated with high yeast and fungal content. In some tanks, including service tanks, fungal material made up a substantial portion of the sludge. Nevertheless, it appeared that well maintained centrifugal purifiers could keep the total volumes of sludge accumulated in service tanks at acceptably low levels.

An ubiquitous particulate contaminant found in all sludges, especially conspicuous in the low-density fraction, was of petroleum origin and is believed to come from oxidation of unstable constituents in diesel fuel. This material was also found on plugged coalescer filters. Much of the reported difficulty with excessive plugging of coalescer filters appeared to be due to taking on off-color fuel containing this kind of petroleum-derived particulate matter. Thus it appears that elimination of microbial growth in fuel storage tanks by the use of a biocide or other agents would not, under present conditions, affect fuel problems caused by this contaminant.

The role of seawater, metals and microorganisms in generating particulate matter by interactions with fuels, especially those prone to instability, needs further investigation.

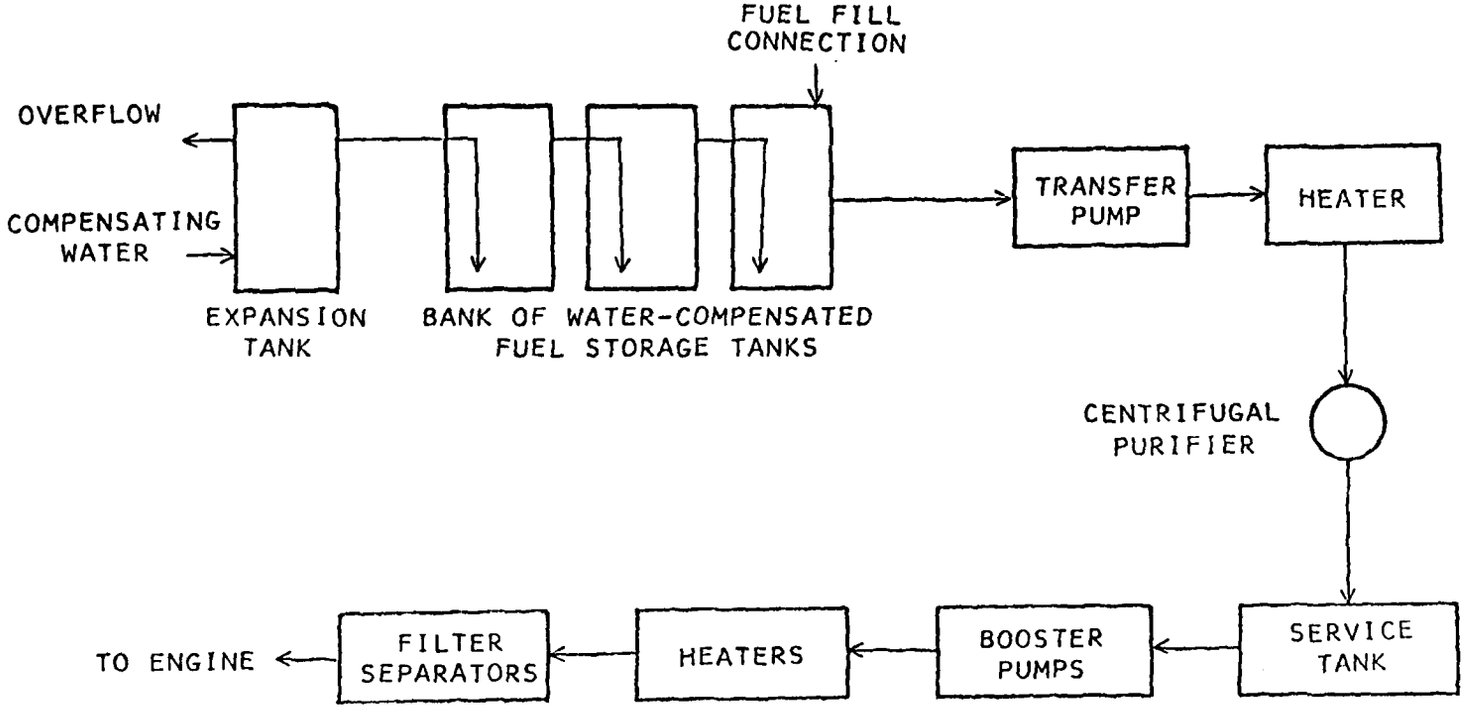

Fig. 1 — Schematic of fuel system on DD-963 class ships

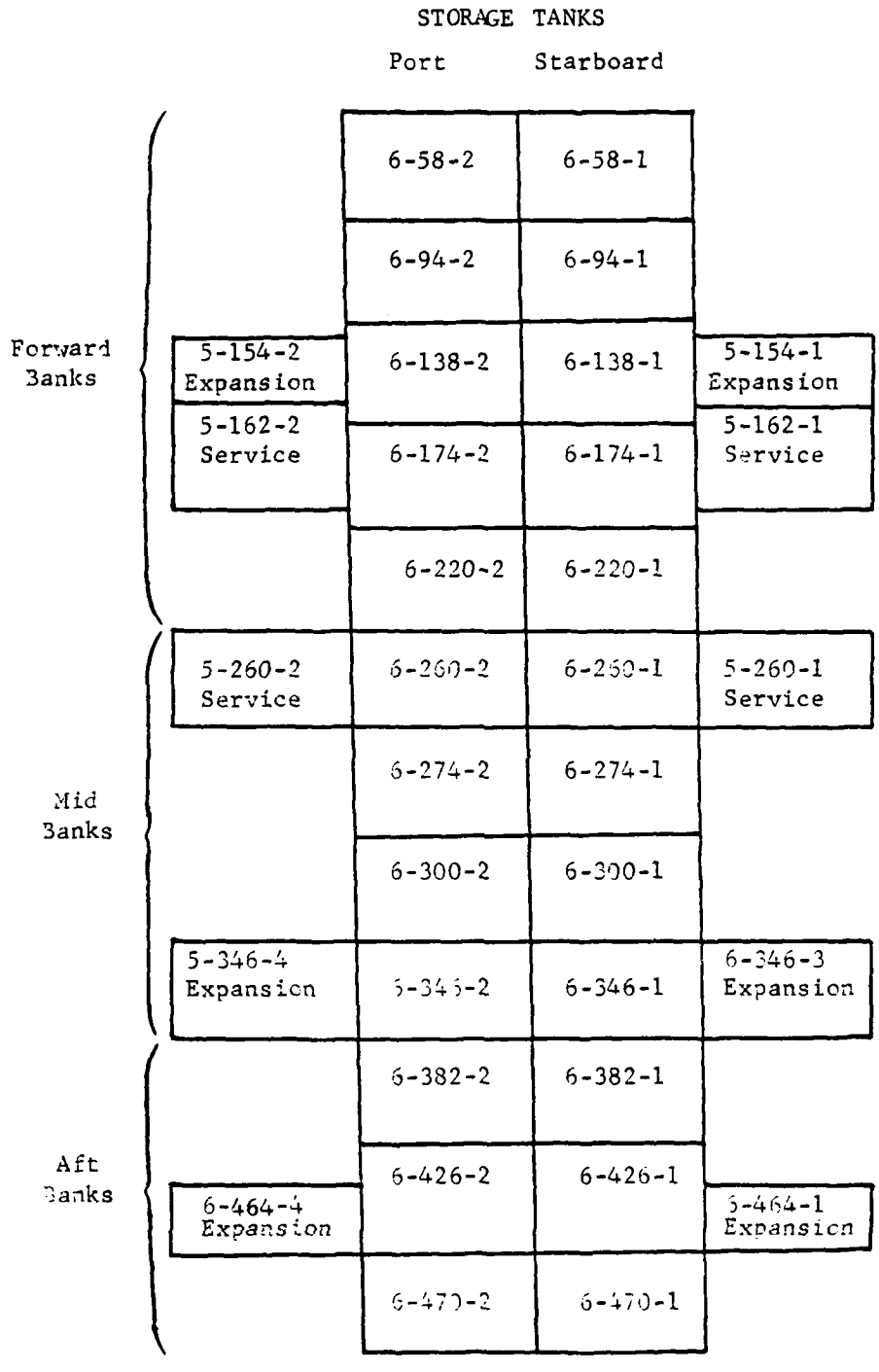

Fig. 2 — Diagram of fuel tank arrangement on DD-963 class ships

Table 1 — Summary of ships sampled

SHIP	SAMPLE DATE	ARRIVAL AT NRL	SUPERVISION	STERILE BOTTLES	NUMBER OF TANKS SAMPLED	OTHER SAMPLES
DD-976	5/79	6/1/79	Ship	no	3	
	4/6-17/81	4/20/81	Ship and NRL	partial	10	
	4/18-28/81	5/22/81	Ship	no	14	
DD-972	9/19/79	8/23/79	Ship	no	0	fuels
	2/25/81	2/26/81	NRL	yes	1 (clean)	coalescers
DD-986	4,5/80	5/2/80	Ship	no	4	coalescer
	4,5/80	6/27/80	Ship	no	11	purifier
DD-992	2/19/81	2/21/81	Ship and NRL	yes	7	
	3/81	3/26/81	Ship	yes	4	
DD-965	2/25/81	2/26/81	NRL	yes	1	
DD-969	3/10/81	3/11/81	NRL	yes	2	
DD-968	9/28,29/81	10/1/81	NRL	yes	22	
DD-967	8/28-9/3/81	11/2/81	Ship	yes	7	

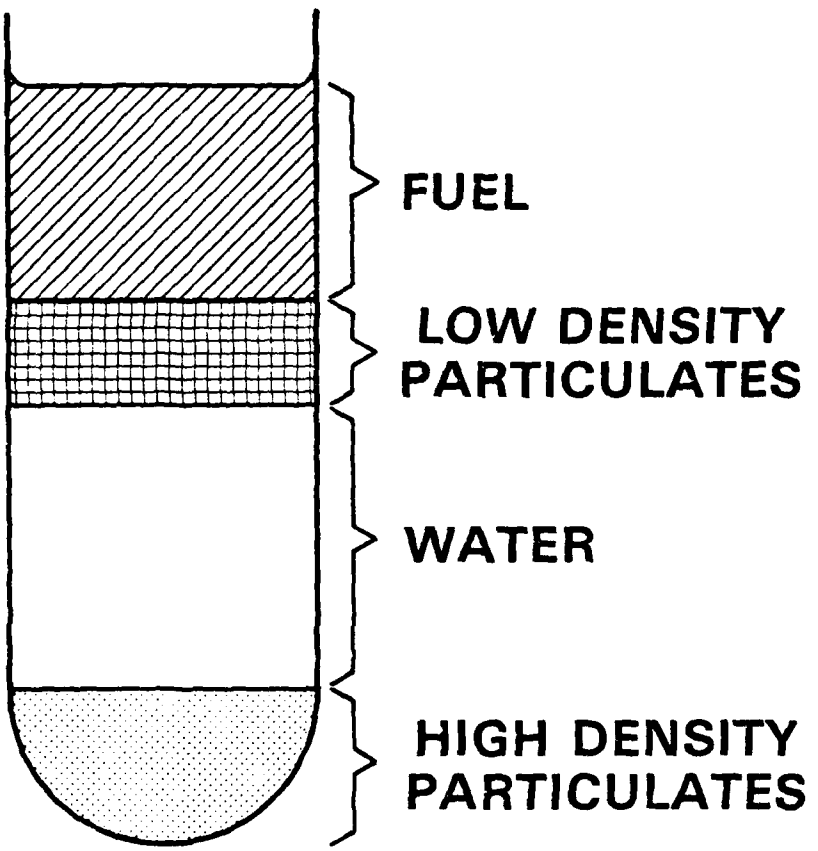

Fig. 3 — Centrifugal fractionation of fuel tank sludge

Tables 2-11

Index of notations:

- , not determined

0 , not detectable

+ , detectable

++ , large amount

ppt , parts per thousand

low , sludge accumulation less than 0.5 cm thick, covering part of the tank bottom

mod., moderate sludge accumulation 0.2 to 0.5 cm covering horizontal surfaces

high , heavy sludge accumulation in excess of 0.5 cm on bottom plus nearly complete coverage on all other surfaces

% , percent by volume for centrifugal fractionation
percent of dry weight for organic

Table 2 — Analyses of fuel tank sludge, DD-976 (5/79)

ANALYSIS	TANK		
	5-154-1	6-138-1	6-174-2
Microbiology:			
microscopic:			
bacteria-	+	++	+
fungi-	0	0	++
viability:			
bacteria-	+	+	+
sulfate-reducers-	++	++	0
fungi-	0	+	+
yeast-	+	+	+
Chemistry:			
sulfide-	++	0	0
pH of water-	7.83	6.92	4.90
salinity of water,ppt.-	35.3	37.0	15.6
Centrifugal fractionation:			
% oil-	4	34	26
% low-density particulates-	4	7	7
% water-	72	46	48
% sediment-	20	13	19
% Organic:			
low-density particulates-	36	58	-
sediment-	15	38	45

Table 3 — Analyses of fuel tank sludge, DD-976 (4/81) (Port Banks)

ANALYSIS	TANK											
	5-260	5-154	6-58	6-174	6-220	6-260	6-274	6-300	6-346	6-464	6-382	6-470
Microbiology:												
microscopic:												
bacteria-	+	+	+	+	+	+	+	+	+	+	+	+
fungi-	+	0	0	0	+	+	+	+	0	0	+	0
viability:												
bacteria-	+	+	+	+	+	+	0	0	+	+	+	+
sulfate-reducers-	0	+	+	+	0	0	0	+	+	+	+	+
fungi-	+	+	0	+	+	+	+	+	+	+	+	0
yeast-	+	0	0	+	+	+	+	+	+	+	+	0
Chemistry												
sulfide-	0	+	+	+	0	0	0	+	+	+	0	+
pH of water-	3.17	8.05	7.67	7.86	4.01	4.03	4.67	7.07	8.00	7.94	5.44	7.38
salinity of water,ppt.	13	33	30	29	40	45	43	39	32	34	40	28
Centrifugal fractionation:												
% oil-	-	55	4	34	45	37	50	38	0	27	37	92
% low-density particulates-	-	6	6	6	16	18	22	19	10	2	11	<1
% water-	-	32	79	48	37	41	23	32	64	69	46	6
% sediment	-	6	12	12	2	4	5	12	26	2	5	3
% Organic:												
low-density particulates-	36	48	42	45	85	70	-	70	47	73	74	100
sediment-	23	28	30	23	40	26	-	43	25	40	25	76

Table 3 (Cont'd) — Analyses of fuel tank sludge, DD-976 (4/81) (Starboard Banks)

ANALYSIS	TANK											
	5-260	5-154	6-58	6-174	6-220	6-346	6-274	6-300	6-260	6-382	6-464	6-470
Microbiology:												
microscopic:												
bacteria-	+	+	-	+	+	+	+	+	+	+	+	0
fungi-	+	0	-	+	+	0	+	+	+	+	0	0
viability:												
bacteria-	+	+	+	+	+	+	+	+	+	+	+	0
sulfate-reducers-	0	+	+	+	+	+	0	+	0	+	-	0
fungi-	+	0	0	+	+	+	+	+	+	+	0	0
yeast-	+	0	0	+	+	+	+	+	+	+	0	0
Chemistry												
sulfide-	0	+	+	+	0	+	0	+	0	0	+	0
pH of water-	3.17	8.11	8.66	7.78	4.59	7.73	4.5	7.34	4.1	6.02	8.00	-
salinity of water,ppt.-	13	34	22.7	33	44	26	41	35	50	39	32	-
Centrifugal fractionation:												
% oil-	31	54	4	12	47	21	47	31	41	34	41	100
% low-density particulates-	15	5	4	3	21	63	20	19	22	11	1	0
% water-	54	33	87	82	30	7	28	40	31	50	54	0
% sediment	9	8	5	2	2	8	5	10	6	5	4	0
% Organic:												
low-density particulates-	86	53	45	53	81	39	93	65	-	68	61	-
sediment-	23	27	35	34	40	23	45	50	-	27	27	-

Table 4 — Analyses of fuels and filter, DD-972 (8/79)

ANALYSIS	SAMPLE					
	Fuel (#1 filter bowl)	Fuel (#2 filter bowl)	Fuel (filter housing)	Fuel (engine inlet)	L.P. filter (pleated paper)	Coalescer (paper)
Microbiology:						
microscopic:						
bacteria-	0	0	0	0	0	0
fungi-	0	+	0	0	0	+
viability:						
bacteria-	+	0	0	0	0	0
sulfate-reducers-	0	0	0	0	0	0
fungi-	+	+	+	+	0	+
yeast-	+	-	-	-	0	-
Chemistry						
sulfide-	0	0	0	0	-	-
Centrifugal fractionation:						
% oil-	100	100	100	100	-	-
% low-density particulates-	-	-	-	-	-	-
% water-	<1	<1	0	0	-	-
% sediment	-	-	-	-	-	-

Table 5 — Analyses of fuel and tank surface, DD-972 (2/81)

ANALYSIS	SAMPLE	
	S-260-2 (Tank)*	Fuel (off-color DFM)
Microbiology:		
microscopic:		
bacteria-	+	+
fungi-	+	0
viability:		
bacteria-	0	0
sulfate-reducers-	-	0
fungi-	+	++
yeast-	+	0
Centrifugal fractionation:		
% oil-	-	99
% low-density particulates-	-	<1
% water-	-	0
% sediment	-	<1
Tank Condition:	Cleaned	-

*surface sample

Table 6 — Analyses of filters and fuel tank sludge, DD-986

ANALYSIS	SAMPLE			
	Coalescer (fiberglass)	Coalescer (resin-bonded paper)	Coalescer (pleated paper)	Purifier
Microbiology:				
microscopic:	-	-	-	++
bacteria-	-	-	-	+
fungi-				
viability:	0	0	0	++
bacteria-	0	0	0	+
sulfate-reducers-	0	+	+	+
fungi-	0	-	-	0
yeast-				
Chemistry	-	-	0	-
sulfide-	-	-	-	6.4
pH of water-	-	-	-	2.9
salinity of water, ppt.-				
% Organic in solid deposit	-	-	90	-

Table 6 (Cont'd) — Analyses of filters and fuel tank sludge, DD-986

ANALYSIS	TANK						
	2-A (Service)	1-B (Service)	2-B (Service)*	2-B (Service)*	6-272-2	6-382-2	6-426-2
Microbiology:							
microscopic:							
bacteria-	+	0	0	0	+	0	+
fungi-	++	0	0	0	+	0	+
viability:							
bacteria-	+	-	+	-	+	-	-
sulfate-reducers-	0	+	0	0	+	0	+
fungi-	+	-	+	-	+	-	-
yeast-	+	-	+	-	+	-	-
Chemistry							
sulfide-	0	++	0	0	+	0	++
pH of water-	4.0	7.6	-	4.5	6.56	4.0	8.2
salinity of water,ppt.-	39.0	34.1	-	43.3	34.2	21.6	22.8
Centrifugal fractionation:							
% oil-	-	-	>99	40	-	38	19
% low-density particulates-	-	-	-	14	-	13	26
% water-	-	-	<1	43	-	46	44
% sediment	-	-	-	3	-	3	11
% Organic:							
low-density particulates-	-	-	-	90	-	77	75
sediment-	-	-	-	40	-	25	30

*Taken at different times

Table 7 — Analyses of fuel tank sludge, DD-992 (2, 3/81)

ANALYSIS	TANK									
	5-162-1	5-260-1	5-154-1	6-58-1	6-34-1	6-174-1	6-260-2	6-346-1	6-346-3	L.I.G.*
Microbiology:										
microscopic:										
bacteria-	+	+	+	+	+	+	0	+	+	+
fungi-	+	+	0	0	0	0	+	0	0	+
viability:										
bacteria-	0	+	+	+	+	+	+	+	+	-
sulfate-reducers-	0	0	+	0	0	0	+	+	+	-
fungi-	+	+	+	0	+	0	+	+	0	+
yeast-	+	+	0	+	+	+	0	+	+	+
Chemistry										
sulfide-	0	0	+	0	0	0	+	0	+	-
pH of water-	3.40	4.02	7.50	7.29	7.34	7.28	6.89	6.89	7.32	-
salinity of water-	30.3	38.1	37.6	44.9	37.6	37.6	31.9	5.5	34.6	-
Centrifugal fractionation:										
% oil-	68	31	0	2.5	0	2	49	24	30	-
% low-density particulates-	13	6	0	1.5	0	2.5	6.5	7.5	1.5	-
% water-	16	63	89	77	79	77	27	42	60	-
% sediment	3	<1	11	19	21	19	17.5	26	9	-
% Organic:										
low-density particulates-	96	70	-	-	-	-	69	64	-	-
sediment-	39	11	25	23	24	22	3	36	17	-

*Level Indicator Guide

Table 8 — Analyses of fuel tank sludge, DD-965

ANALYSIS	Tank 5-154-2
Microbiology:	
microscopic:	
bacteria-	+
fungi-	0
viability:	
bacteria-	+
sulfate-reducers-	++
fungi-	+
yeast-	+
Chemistry	
sulfide-	0
pH of water-	7.56
salinity of water,ppt.-	36.9
Centrifugal fractionation:	
% oil-	12
% low-density particulates-	1
% water-	85
% sediment	2
% Organic:	
low-density particulates-	39
sediment-	30
Sludge accumulation:	mod.

Table 9 — Analyses of fuel tank sludge, DD-969

ANALYSIS	TANK 5-260-2	TANK 6-272-2
Microbiology:		
microscopic:		
bacteria-	++	+
fungi-	++	+
viability:		
bacteria-	0	+
sulfate-reducers-	0	++
fungi-	+	+
yeast-	+	+
Chemistry		
sulfide-	0	0
pH of water-	4.0	7.2
salinity of water,ppt.-	7.8	37.2
Centrifugal fractionation:		
% oil-	85	18
% low-density particulates-	5	15
% water-	7	59
% sediment	4	8
% Organic:		
low-density particulates-	91	55
sediment-	22	37
Sludge Accumulation:	low	high

Table 10 — Analyses of fuel tank sludge, DD-968 (Starboard Banks)

ANALYSIS	TANK											
	5-162	5-154	6-58*	5-94*	6-174*	6-220	6-260	6-272	6-300	6-346	6-382	6-426
Microbiology:												
microscopic:												
bacteria-	+	+	+	+	+	+	+	+	+	+	+	+
fungi-	+	+	0	+	+	+	+	+	+	+	+	+
viability:												
bacteria-	+	+	+	+	+	+	0	+	+	+	+	+
sulfate-reducers-	0	++	+	+	+	+	0	+	+	+	++	++
fungi-	+	+	+	+	+	+	+	+	+	+	+	+
yeast-	+	+	+	+	+	+	+	0	0	+	+	+
Chemistry												
sulfide-	0	+	0	0	0	+	0	0	0	0	0	+
pH of water-	3.3	8.1	-	7.5	7.3	5.3	3.9	5.2	5.6	7.5	6.3	7.3
salinity of water-	37.5	38.0	38.6	28.4	25.5	40.5	45.7	45.5	44.5	40.2	37.2	31.4
Centrifugal fractionation:												
% oil-	41	15	22	-	-	25	22	30	34	33	27	31
% low-density particulates-	13	6	8	-	-	26	19	23	15	16	25	24
% water-	44	55	44	-	-	45	57	40	35	38	37	37
% sediment-	2	23	26	-	-	5	2	7	15	13	11	8
% Organic:												
low-density particulates-	65	49	52	-	-	75	75	58	63	60	69	70
sediment-	41	10	40	-	-	31	25	33	40	31	24	39
Sludge accumulation:	low	mod.	mod.	cleaned	mod.	mod.	high	high	mod.	mod.	mod.	mod.

*small sample

Table 10 (Cont'd) — Analyses of fuel tank sludge, DD-968 (Port Banks)

ANALYSIS	TANK									
	5-162	5-260	5-154	6-94*	6-174**	6-220	6-346	6-260	6-272	6-300
Microbiology:										
microscopic:										
bacteria-	+	+	+	+	+	+	+	+	+	+
fungi-	+	+	+	+	+	+	+	+	+	+
viability:										
bacteria-	+	0	+	+	+	0	+	0	+	+
sulfate-reducers-	0	0	++	++	0	0	+	0	+	+
fungi-	+	+	+	+	+	+	+	+	+	+
yeast-	+	+	+	+	+	+	+	+	0	+
Chemistry										
sulfide-	0	0	+	+	-	+	+	0	+	0
pH of water-	3.4	3.2	8.5	7.7	-	4.0	7.2	3.8	5.6	7.1
salinity of water, ppt.-	32.6	27.2	33.1	35.3	-	>60	40.7	58.5	49.7	45.5
Centrifugal fractionation:										
% oil-	45	44	36	-	-	16	23	21	27	22
% low-density particulates-	17	17	5	-	-	36	16	3	35	8
% water-	29	37	43	-	-	47	40	47	37	55
% sediment	8	2	16	-	-	1	21	8	10	15
% Organic:										
low-density particulates-	96	90	50	-	-	64	30	77	60	57
sediment-	57	3	19	-	-	75	28	43	40	23
Sludge accumulation:	low	low	mod.	-	low	mod.	mod.	high	high	mod.

*Fuel/water sample, few particulates
**Small sample

Table 11 — Analyses of fuel tank sludge, DD-967

ANALYSIS	TANK						
	5-162-1	5-162-2	5-260-1	5-260-2	6-58-1	6-260-1	6-260-2
Microbiology:							
microscopic:							
bacteria-	+	+	++	++	+	+	+
fungi-	+	+	+	++	+	+	+
viability:							
bacteria-	0	0	+	++	++	++	++
sulfate-reducers-	0	0	0	0	+	+	0
fungi-	+	+	+	+	+	+	+
yeast-	++	++	++	+	++	++	++
Chemistry							
sulfide-	0	0	-	-	+	+	+
pH of water-	-	-	4.17	4.17	6.33	6.38	5.97
salinity of water,ppt.-	-	-	11.7	13.2	39.3	30.6	37.1
Centrifugal fractionation:							
% oil-	96	99	55	95	31	26	38
% low-density particulates-	4*	0	9	<1	19	14	17
% water-	0	0	34	4	43	55	36
% sediment	0	<1	2	<1	8	5	9
% Organic:							
low-density particulates-	78*	-	86	-	79	67	74
sediment-	-	-	52	-	23	31	22

*Includes sediment

Fig. 4a — Interior of a fuel tank with low contamination.
The tank shown is a service tank.

Fig. 4b — Interior of a fuel tank with moderate contamination. The tank shown is a storage tank.

Fig. 4c — Interior of a fuel tank with high contamination.
The tank shown is a storage tank.

Fig. 5 — Fuel tank sludges characterized as slimy and jelly-like (top) and coarse and granular (bottom)

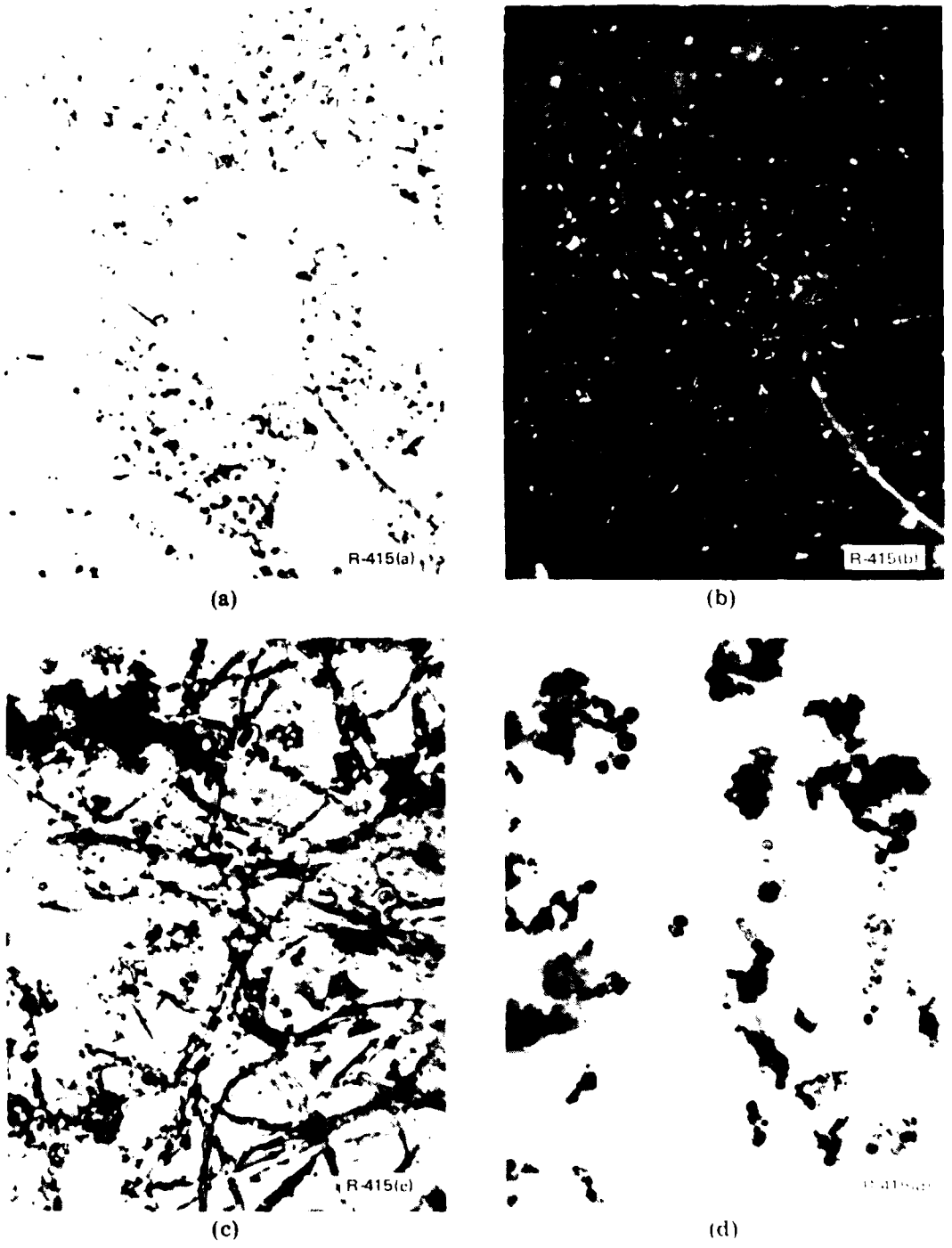

Fig. 6 — Photomicrographs of different sludge materials: (a) phase contrast view of sludge dominated by bacteria; (b) same field by epifluorescence with acridine orange stain; (c) sludge dominated by fungi and yeast; (d) particles characteristic of unstable diesel fuel. Scale: 1 mm = 1 μm.

Table 12 — Microorganisms most frequently found in sludges from fuel storage tanks on DD-963 class ships

Fungi and yeast	Bacteria
Candida	Pseudomonas
Paecilomyces	Desulfovibrio
Cladosporium resinae	
Unidentified small budding yeast	
Fusarium	
Aspergillus	
Penicillium	
Mucorales	
Sterile white fungi	

Table 13 — Summary of microbiological characteristics of fuel tank sludges associated with differences in aqueous pH

		pH > 7.8	pH = 4 - 7.8	pH < 4
1.	Predominate character	Bacteria	Yeast and variety of fungi	Yeast and C. resinae
2.	Sulfate reducers	Usually positive	Often positive	Negative
3.	Sulfide	Often positive	Rarely positive	Negative
4.	Bacteria	Wide variety Very numerous	Variety Not as numerous	Few
5.	Fungi	Wide variety Not numerous	Variety Numerous	Mainly C. resinae Numerous
6.	Yeast	Few	Mainly Candida Very numerous	Mainly Candida Very numerous
7.	Enrichment cultures fuel as C-source	Low growing or negative	Fast growing with different fungi predominating	Fast growing with C. resinae predominating

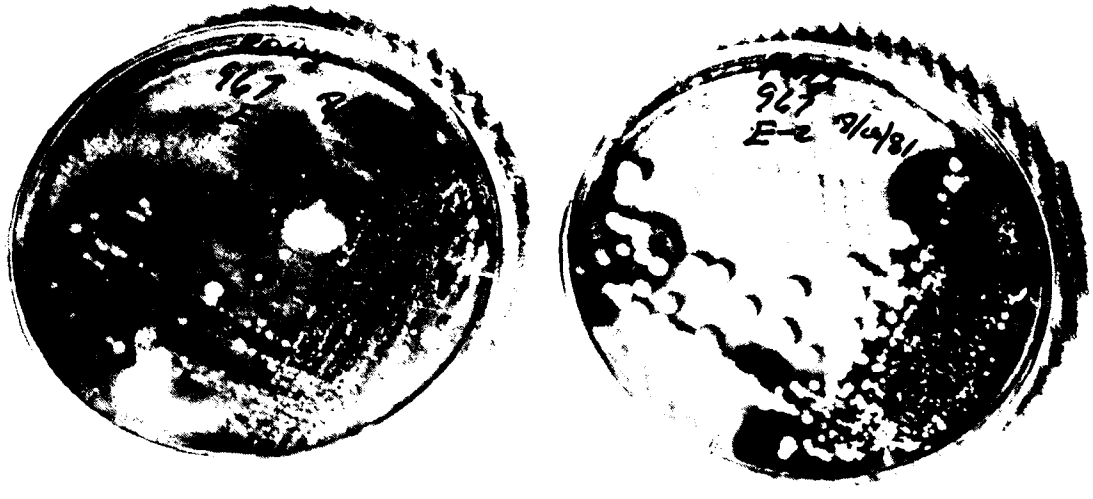

Fig. 7 — Agar plates showing microbial growth from different fuel tank sludges: (left) a variety of fungi and bacteria associated with an intermediate pH (6.3); (right) two species of fungi, including *C. resinae* (dark areas), found in sludge with an aqueous pH less than 4.

Table 14 — Analyses of dockside water samples

Analysis	Pascagoula, MS 5/5/80	Pascagoula, MS 2/19/81	San Diego, CA 2/25/81	Norfolk, VA 3/10/81
pH	6.11	7.0	7.77	8.05
Salinity, ppt	2.9	6.1	35.3	22.6
Microscopic examination	Bacteria	Bacteria	Bacteria	Bacteria
Viability	Bacteria	Bacteria Yeast	Bacteria Yeast	Bacteria
Sulfate reducers	+	+	+	+
Growth on fuel as a carbon source	Bacteria	Bacteria	Bacteria	No growth

Table 15 — Microbial growth from sludge inocula in diesel fuel/seawater media (enrichment cultures)

Ships/Organisms	Tanks*											
DD-976	5-260-1	5-154-1	6-58-1	6-174-1	6-220-1	6-260-1	6-274-1	6-300-1	6-346-1	6-464-1	6-382-1	6-470-1
Bacteria	0	+	+	+	+	0	0	+	+	+	+ 0	
Fungi (yeast)	+	+	+	+	+	+	+	+	+	+	+ 0	
	5-260-2	5-154-2	6-58-2	6-174-2	6-220-2	6-260-2	6-274-2	6-300-2	6-346-2	6-464-2	6-382-2	6-470-2
Bacteria	0	+	+	+	+	0	0	0	+	+	+ 0	
Fungi (yeast)	+	0	+	+	+	+	+	+	+	+	+ +	
DD-972	5-260-2											
Bacteria	0											
Fungi (yeast)	+											
DD-992	5-162-1	5-260-1	5-154-1	6-58-1	6-94-1	6-174-2	6-260-2	6-346-1	6-346-3			
Bacteria	0	0	+**	+	+	+	0	0	+**			
Fungi (yeast)	+	+	+	+	+	+	+	+	+			
DD-965	5-154-2											
Bacteria	+											
Fungi (yeast)	+											
DD-969	5-260-2	6-272-2										
Bacteria	+**	0										
Fungi (yeast)	+	+										
DD-968	5-162-1	5-154-1	6-58-1	6-94-1	6-174-1	6-220-1	6-260-1	6-272-1	6-300-1	6-346-1	6-382-1	6-426-1
Bacteria	0	+**	+	+	+	+	0	+	+**	+	+ +**	
Yeast	+	+	+	+	+	+	+	0	+	+	+ 0	
Fungi	+	+	0	0	+	+	+	+	+	+	+ 0	
DD-968	5-162-2	5-154-2	5-260-2	6-94-2	6-174-2	6-220-2	6-260-2	6-272-2	6-300-2	6-346-2		
Bacteria	0	+**	0	+**	+	0	+	0	+	+**		
Yeast	+	+	+	+	+	+	+	0	+	+		
Fungi	+	0	+	0	+	0	+	+	+	+		
DD-967	5-162-1	5-162-2	5-260-1	5-260-2	6-58-2	6-260-1	6-260-2					
Bacteria	0	0	0	0	+	+	+					
Yeast	+	+	+	+	+	+	+					
Fungi	+	+	+	+	+	+	+					
DD-967	5-162-1	5-162-2	5-260-1	5-260-2	6-58-2	6-260-1	6-260-2					
Bacteria	0	0	0	0	+	+	+					
Yeast	+	+	+	+	+	+	+					
Fungi	+	+	+	+	+	+	+					

* - 0 - No growth detected
\+ - Detectable growth

** - sulfate-reducing bacteria present

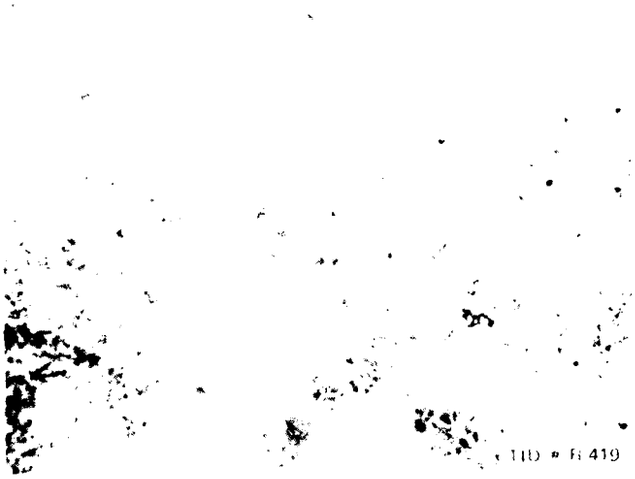

Fig. 8 — Phase contrast view of residue from guide rod of fuel level indicator showing fungal fragments, petroleum particulates and other debris. Scale 1 mm = 1 μm.

Table 16 — Major metals in sludge fractions after water wash and incineration

Ship/Tank	Low-density particulates (μg/mg)	Sediment (μg/mg)
DD-986		
2A (Service)	Fe(120),Ba(14),Cu(3.4)	Fe(211),Zn(28),Cu(25)
2B (Service)	Fe(157),Ni(35),Zn(19)	Fe(272),Zn(11),Cu(9.7)
Purifier	Fe(229),Ni(26),Zn(19)	Fe(570),Cu(11),NI(4)
DD-976		
5-260-1	Fe(42.3),Cu(2.8),Ni(1.6)	-
5-260-2	Fe(41.2),Mg(10.4),Cu(3.2)	-
5-154-1	-	Fe(15.1),Ca(8.0),Cu(3.7)
5-154-2	Fe(20),Zn(16),Cu(12)	Ca(19.2),Fe(8.1),Zn(5.3)
6-58-1	-	Zn(12.1),Fe(11.6),Cu(4.1)
6-58-2	-	Fe(25.6),Cu(15.6),Ni(3.7)
6-174-1	-	Fe(13.1),Al(3.2),Cu(2.6)
6-174-2	-	Fe(17.3),Ca(3.6),Ni(2.9)
6-220-1	Fe(50.5),Ni(3.3),Mg(2.5)	-
6-220-2	Fe(40),Cu(18),Ni(7.3)	Fe(60),Zn(3.6),Cu(3.5)
6-382-1	Fe(46),Cu(2.8),Ni(1.8)	-
6-382-2	Fe(54),Cu(4.4),Ni(3.5)	Fe(62),Cu(1.1)
6-464-2	-	Zn(20.6),Fe(11.5),Ca(1.1)
DD-968		
5-162-1	Fe(19.3),Cu(0.53)	Fe(41),Ba(1.3)
5-162-2	Fe(24.8),Ni(1.2),Zn(1.0)	Fe(46),Ba(1.4)
5-260-2	Fe(43),Ni(3.0),Cu(1.7)	Fe(60),Ba(0.5)

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APPENDIX A

UNITED STATES GOVERNMENT

memorandum

6130-38:RP:blt

DATE: 23 March 1982

REPLY TO ATTN OF: Code 6133 (Rm. Panayappan)

SUBJECT: Chemical Analyses of Sludge Samples from Ship Fuel Tanks of USS Merrill and USS Radford

TO: Code 4353 (R. Neihof)

Ref: (a) Conversation between R. Neihof and Rm. Panayappan (10/15/81)
(b) 61-1379-A-1

Encl: (1) Chemical Analyses of Sludge Samples from Ship Fuel Tanks

1. In connection with reference (a) and (b) enclosure (1) is forwarded for your information and retention.

Rm. Panayappan
Photochemistry Unit
Inorganic and Electrochemistry Branch
Chemistry Division

OPTIONAL FORM NO. 10
(REV. 1-80)
GSA FPMR (41 CFR) 101-11.6
5010-114

☆U.S. Government Printing Office: 1980—341-526/6193

ANALYSES OF SLUDGE SAMPLES FROM SHIP FUEL TANKS

Experimental

Twenty-two powdered samples of incinerated sludge material from three ships, DD-976, DD-968 and DD-986, were received from R. Neihof.

Weighed quantities of the samples were suspended in 10 ml of aqua regia in separate beakers and heated on a hot plate at 70°C until all the acid evaporated. The beakers were cooled, 5 ml of conc. HNO_3 were added and the solution was diluted to 100 ml with reagent water in volumetric flasks. A small portion of each sample did not dissolve.

DC argon plasma atomic emission multielement analyses were carried out for nineteen elements.

Results and Discussion

The results obtained are tabulated in four tables (the relative standard deviation is $\leq$ 5%, ref. 1). Iron and copper are the predominant metals while the other metals Zn, Mg, Ca, Al and Ni are also present in appreciable concentrations. The results of Tables 1 and 2 indicate the presence of appreciably more magnesium and nickel in low-density particulate samples than in sediment samples of DD-976.

Four samples from the volumetric flasks were filtered through a 0.45 HA Millipore filter, dried in a desiccator and weighed. Table A summarizes the data obtained for representative samples. The total weight percent of the nineteen elements and the residue is not equal to 100%. Iron and other metals are probably existing in the form of oxides (Fe_2O_3 or Fe_3O_4) in these samples. Calculated percentage of oxygen in Fe_2O_3 is 30% and Fe_3O_4 is 28%. This difference, of approximately 30%, found in Table A, may be attributed to oxygen present in these metal oxides.

X-ray fluorescence analyses of the residues of the above four samples showed relatively stronger peaks for silicon and titanium than for iron and sulfur. Tanks 6-174-2 (DD-976) and 5-162-1 (DD-968) showed relatively more intense peaks than the other two samples supporting the lesser insoluble material found in 6-220-2 (DD-976) and 5-260-2 (DD-968).

Conclusion

From tables A, and 1-4, it appears that all these solids are mixtures of metal oxides and sand particles.

Reference

1. "The Determination of Detection Limits by DC Argon Plasma Atomic Emission Spectrometry," Suzanne K. Smidt, Rm. Panayappan and J.C. Cooper, NRL Memorandum Report 4583, July 15, 1981. Relative standard deviations were obtained using at least three analysis runs on each sample.

Table A

SHIP	TANK	FRACTION	WEIGHT PERCENT Residue	Fe	Cu	Other Metals	Total
DD-976	6-174-2	Sediment	40	17.3	1.2	12.9	71.4
DD-976	6-220-2	Low Density	5	40.3	18.3	15.0	78.6
DD-968	5-260-2	Sediment	6	60.1	0.1	1.9	68.1
DD-968	5-162-1	Low Density	44	19.0	0.5	3.0	63.8

Table 1 — DC argon plasma atomic emission analysis of metals in incinerated sludges from fuel tanks DD-976 (4/81) sediment fraction

Element	Tank (wt - %)								
	5-154-1	5-154-2	6-58-1	6-58-2	6-174-1	6-174-2	6-220-2	6-282-2	6-464-2
B	0.07	BDL	BDL	BDL	0.04	0.06	0.04	0.02	0.13
P	0.14	0.16	0.18	0.13	0.19	0.16	0.35	0.12	0.29
Cd	BDL	BDL	0.01	BDL	BDL	BDL	BDL	BDL	0.02
Fe	15.11	8.11	11.61	25.56	13.14	17.25	60.37	61.94	11.48
Zn	5.63	5.29	12.10	2.06	1.36	2.63	3.64	0.48	20.56
Hg	BDL	0.06	BDL	BDL	BDL	BDL	0.06	0.04	0.01
Cu	3.67	3.69	4.13	15.62	2.63	1.15	3.45	1.14	BDL
Mg	1.46	2.3	0.76	0.71	1.23	0.88	0.41	0.19	0.74
Mn	BDL	BDL	0.01	0.03	0.04	0.03	0.23	0.29	0.03
Pb	BDL	0.04	0.01	BDL	BDL	BDL	0.22	0.03	0.06
Ca	8.03	19.16	2.18	2.72	0.83	3.61	0.31	0.16	1.09
Co	0.03	0.08	0.01	BDL	BDL	BDL	BDL	BDL	0.04
Al	1.07	1.52	1.24	2.07	3.17	1.53	1.03	0.25	0.14
Sn	BDL	0.04	BDL	BDL	0.11	0.13	2.52	0.13	0.05
Ba	BDL	0.01	BDL	0.04	BDL	BDL	0.46	0.04	0.02
Ni	1.18	0.86	1.29	3.72	0.90	2.90	0.93	0.48	1.04
Cr	BDL	0.02	BDL	BDL	BDL	BDL	0.46	0.06	0.04
Li	0.04	0.07	BDL	BDL	BDL	0.01	BDL	BDL	0.04
V	0.02	0.04	BDL	BDL	BDL	BDL	BDL	BDL	0.04

BDL - Below detection limits (ref. 1)

Table 2 — DC argon plasma atomic emission analysis of metals in incinerated sludges from fuel tanks, DD-976 (4/81), low-density particulate fraction

Element	Tank (wt - %)						
	5-260-1	5-260-2	5-154-2	6-220-2	6-220-3	6-382-1	6-382-2
B	0.03	BDL	0.02	BDL	BDL	BDL	BDL
P	0.17	0.24	0.55	0.03	0.29	0.12	0.03
Cd	0.02	0.03	BDL	BDL	BDL	BDL	BDL
Fe	42.33	41.22	20.1	40.33	50.46	45.62	53.61
Zn	0.42	0.88	16.1	1.58	1.39	1.96	1.32
Hg	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Cu	2.79	3.22	11.83	18.31	2.04	2.76	4.42
Mg	0.30	10.39	1.86	2.34	2.51	1.21	1.73
Mn	0.10	0.11	0.04	0.24	0.42	0.12	0.22
Pb	0.10	0.15	0.07	0.15	0.21	0.29	0.06
Ca	0.37	0.46	4.81	2.40	2.22	1.16	1.87
Co	BDL	0.03	0.04	BDL	BDL	BDL	BDL
Al	0.21	0.20	1.32	0.70	1.52	0.43	1.16
Sn	0.23	0.27	0.02	0.06	BDL	0.41	BDL
Ba	0.55	0.45	0.02	0.66	0.46	0.43	0.72
Ni	1.58	1.85	1.30	7.26	3.28	1.75	3.46
Cr	BDL	0.29	0.03	0.01	0.73	0.07	0.88
Li	BDL	BDL	0.04	0.01	BDL	0.03	0.04
V	BDL	BDL	0.01	0.06	BDL	0.03	0.04

BDL - Below detection limits (ref. 1)

Table 3 — DC argon plasma atomic emission analysis of metals in incinerated sludges from fuel tanks, LD-968

Element	Tank (wt - %)					
	Sediment Fraction			Low-Density Fraction		
	5-162-1	5-162-2	5-260-2	5-162-1	5-162-2	5-260-2
B	0.08	0.08	0.06	0.09	BDL	1.00
P	0.12	0.10	0.14	0.12	0.19	0.90
Cd	BDL	BDL	BDL	BDL	BDL	BDL
Fe	40.98	45.51	60.13	19.27	24.81	43.02
Zn	0.13	0.29	0.13	0.51	1.03	1.21
Hg	0.02	BDL	0.10	BDL	BDL	BDL
Cu	0.40	0.50	0.10	0.53	0.94	1.65
Mg	0.24	0.21	0.10	0.29	0.46	0.08
Mn	0.31	0.33	0.33	0.11	0.12	0.22
Pb	0.09	0.10	0.02	0.11	BDL	0.02
Ca	0.19	0.50	0.10	0.31	0.54	1.15
Co	0.01	BDL	BDL	0.01	BDL	BDL
Al	0.38	0.24	0.10	0.31	0.64	0.51
Sn	0.01	BDL	0.10	BDL	BDL	BDL
Ba	1.27	1.37	0.51	0.27	0.15	0.44
Ni	0.37	0.36	0.18	0.12	1.23	3.02
Cr	0.31	0.22	BDL	0.08	0.24	1.00
Li	0.01	BDL	0.1	BDL	0.2	1.00
V	0.01	BDL	BDL	BDL	0.03	BDL

BDL - Below detection limits (ref. 1)

Table 4 — DC argon plasma atomic emission analysis of metals in incinerated sludges from fuel tanks, DD-986

Element	Tank (wt - %)					
	Sediment Fraction			Low-Density Fraction		
	2-A		2-B	2-A		2-B
	Service	Purifier	Service	Service	Purifier	Service
B	0.03	0.03	BDL	BDL	0.05	0.03
P	0.43	0.22	0.34	0.12	0.32	0.27
Cd	0.04	0.03	0.07	0.01	0.05	0.07
Fe	21.13	56.99	27.24	12.04	22.93	15.67
Zn	2.78	0.36	1.11	0.21	0.68	1.86
Hg	BDL	BDL	BDL	BDL	BDL	BDL
Cu	2.46	1.09	0.97	0.34	1.73	0.91
Mg	1.00	0.17	0.55	0.22	1.18	1.63
Mn	0.14	0.25	0.07	0.12	0.14	0.13
Pb	0.01	0.11	0.21	0.11	0.05	0.07
Ca	1.43	0.14	0.34	0.22	0.82	1.13
Co	0.01	0.01	0.05	0.01	0.02	0.02
Al	0.04	0.33	0.55	0.14	0.04	0.17
Sn	0.01	BDL	BDL	0.10	0.01	-
Ni	1.07	0.41	0.55	0.09	2.64	3.53
Cr	0.03	0.03	0.03	0.02	0.01	0.03
Li	0.01	BDL	0.01	BDL	0.01	0.02
V	BDL	BDL	BDL	BDL	0.01	0.01
Ba	0.02	0.02	0.02	1.37	0.27	0.07

BDL - Below detectin limits (ref. 1)

All of above samples show presence of carbon, sodium, tungsten and silicon by qualitative Echelle Prism Spectrogram.

END

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